

## Color-pattern modifications and speciation in lycaenid butterflies

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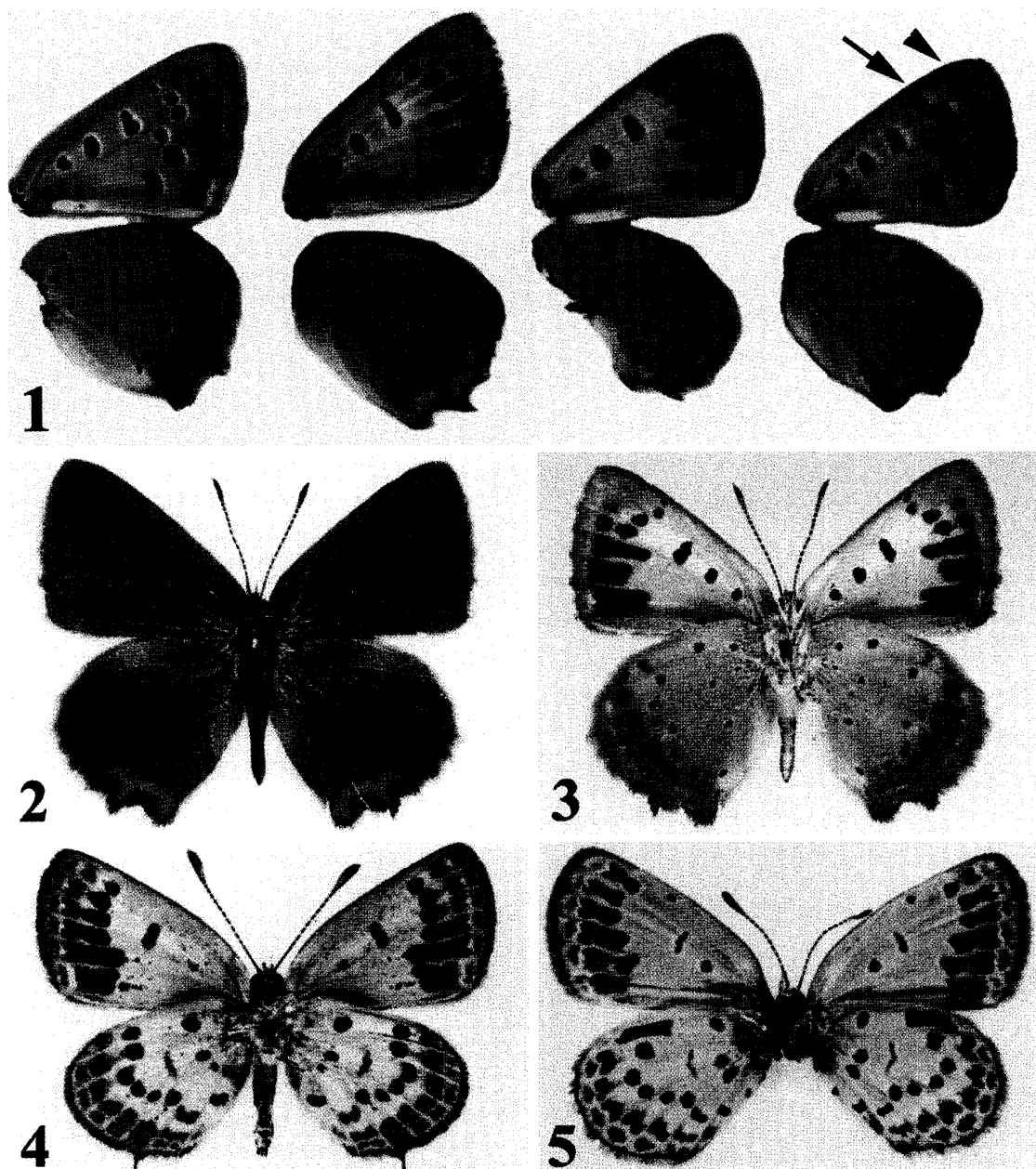
**Abstract** We have previously shown that the systemic injection of sodium tungstate, a protein-tyrosine phosphatase (PTPase) inhibitor, to pupae immediately after pupation efficiently produces characteristic color-pattern modifications on the wings of the Painted Lady butterfly *Vanessa cardui*. In this species, the black spots reduced in size and eventually disappeared in response to the treatment. The observed modifications probably resulted from the prolonged activation of a signal transduction cascade initiated by a receptor-type protein-tyrosine kinase in scale cells. Here we observed that applying this method to the Small Copper butterfly *Lycaena phlaeas daimio*, the black spots in the ventral wings enlarged in size, which was in sharp contrast to the induced modifications in *V. cardui*. This type of modifications seen in *L. phlaeas daimio* has frequently been reported in many field-caught individuals of Lycaenidae. By simply examining the natural color-patterns of Japanese lycaenid butterflies, we here note that the enlargement of spots is related with speciation in this family. The most remarkable example can be found in the Japanese *Maculinea* species, *M. teleius* and *M. arionides*. While the former has small spots, the latter has “enlarged” spots in the ventral wings, which is reminiscent of the tungstate-treated modifications in *L. phlaeas daimio*. Since it has been proposed that a hypothetical transduction pathway with a PTPase for the scale-cell differentiation globally coordinates the wing-wide color-patterns, our findings suggest that spontaneous mutations in genes in this hypothetical pathway might have played a role in creating new color-patterns and species at least in Lycaenidae.

**Key words** *Lycaena phlaeas daimio*, *Maculinea teleius*, *Maculinea arionides*, protein-tyrosine phosphatase, protein-tyrosine kinase, sodium tungstate, color-pattern modifications, speciation, lycaenid butterflies.

## Introduction

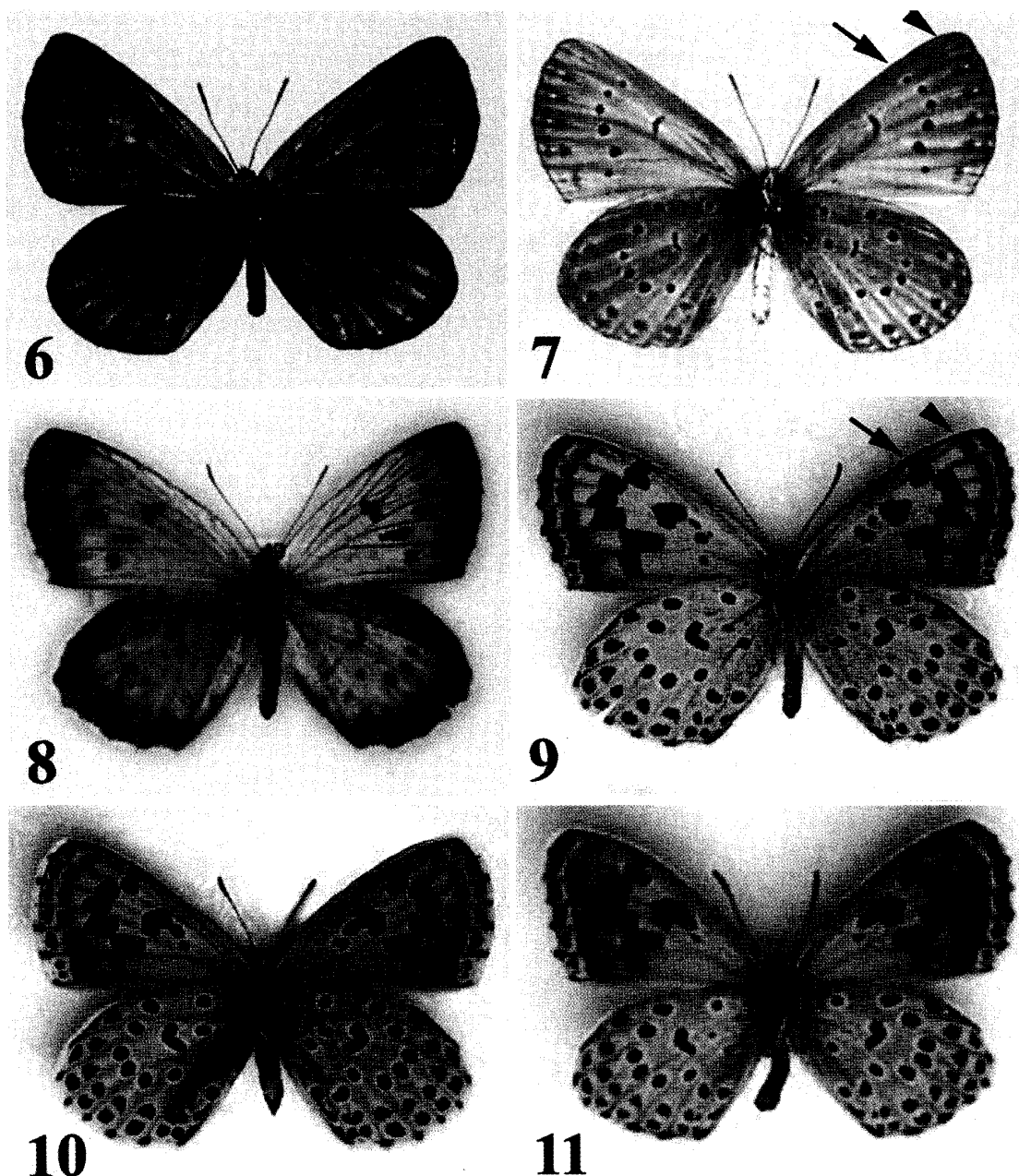
A wide variety of the Lepidopteran wing color-patterns is probably one of the most diverse phenotypes among all organisms. This is largely because the color patterns on the wing surfaces are conspicuous traits for natural selection by predators (Brakefield, 1987; Uesugi, 1991; Grant *et al.*, 1996; Brakefield & Liebert, 2000; Kapan, 2001) and mates (Jiggins *et al.*, 2001; Sweeney *et al.*, 2003). Butterfly wings thus offer an excellent system to investigate from the viewpoint of evolutionary and developmental biology how these various morphogenic traits have been invented and maintained in nature (Nijhout, 1991; Brakefield *et al.*, 1996; Brunetti *et al.*, 2001; Beldade & Brakefield, 2002).

In addition to various natural forms of butterflies from around the world, many aberrant forms of butterflies caught in the field have been reported in lepidopterology journals. Some of these “spontaneous” aberrant color-patterns have been systematically reviewed in the light of wing homeosis (Sibatani, 1980, 1983*a*, 1983*b*). Recent advances in molecular genetics and developmental biology of the fruit fly made it possible to understand to some extent molecular events leading to these aberrant forms (Warren *et al.*, 1994). However, some other aberrant types of butterflies remain to be examined systematically.



Figs 1-5. Artificially-induced or spontaneous modifications of color-pattern elements in Lycaenidae. 1. Modifications induced by the tungstate treatment in *L. phlaeas daimio*. The left-most one is a non-treated individual showing a natural color-pattern. Others show modified patterns, although their precise patterns of modifications were different from individual to individual. An arrow and an arrowhead indicate Media 1 (distal band) and Externa 3 (element g), respectively, which are spot lines that are highly sensitive to this treatment. 2 and 3. A field-caught individual of *L. phlaeas daimio* with modifications that are similar to the artificially-induced ones (Miyakuni, 1991). Reproduced from *Chouken Field*. 4 and 5. Field-caught aberrant individuals of *Tongeia fischeri* (Fujiwara, 1992) and *Pseudozizeeria maha argia* (Tsunemoto, 1988). Reproduced from *Chouken Field*.

Since more than a century ago, many researchers such as Standfuss in Germany and Merrifield in England have tried to produce aberrant forms in artificial measures (Sakaguchi, 1981). It has been revealed that temperature shock at the late larval and pupal stages could produce



Figs 6-11. Comparison of color patterns between two *Maculinea* species, *M. teleius* and *M. arionides*. 6 and 7. *M. teleius*, dorsal and ventral sides. An arrow and an arrowhead indicate spot lines, Media 1 and Externa 3, respectively. These spot lines correspond to those of Figs 1 and 9. This specimen was provided by Dr Masatoshi Takakuwa, Kanagawa Prefectural Museum of Natural History. 8-11. *M. arionides*, dorsal and ventral sides. These specimens were provided by Mr Takuya Ito and Mr Ryo Takahashi in Hokkaido University.

characteristic wing-pattern modifications with fuzzy pattern boundaries in many species of butterflies and moths (Sakaguchi, 1981). More systematic studies on the temperature-shock-induced modifications of the butterfly wing color-patterns have been performed by Nijhout (1984, 1985a) using *Vanessa cardui*, *Vanessa virginiensis*, and *Precis coenia*, all of which belong to the subfamily Nymphalinae of the family Nymphalidae. In these species, the induced aberrant patterns can be arranged in a linear progressive series according to

modification degrees (Nijhout, 1984, 1985*a*). These modified individuals are considered to be “phenocopies” that are equivalent to *Drosophila* ones (Nijhout, 1984, 1985*a*, 1991).

Following this work, we have previously shown that using *V. cardui* the “cold-shock property” can be transferred from the cold-shocked to the non-cold-shocked individuals *via* hemolymph transfusion (Otaki, 1998). This immediately suggests the existence of a “cold-shock hormone” that coordinates many pattern elements throughout wings and further suggests the existence of chemical substances that can mimic the activity of this putative hormone when injected into pupae (Otaki, 1998).

Accordingly, we have demonstrated that the systemic injection of some oxyanions, especially sodium tungstate, can induce the color-pattern modifications that are very similar to those induced by the cold-shock treatment (Otaki, 1998, 2003). High efficiency of the tungstate treatment is illustrated in the fact that in *V. cardui* the tungstate injection induced some modifications in 100% and the most extreme modification in 63% of the treated individuals (Otaki, 1998), whereas the optimized cold-shock treatment induced some modifications in about 60% and the most extreme modification only in 2.6% (Nijhout, 1984). Thus, it is reasonable to speculate that tungstate mimics the action of the putative cold-shock hormone. Since tungstate is a well-known competitive inhibitor for protein-tyrosine phosphatases (PTPases) at the cellular and animal physiology level (Fillat *et al.*, 1992; Goto *et al.*, 1992; Barbera *et al.*, 1994; Matsumoto, 1994; Haque *et al.*, 1995; Li *et al.*, 1995) as well as at the crystal structure level (Stuckey *et al.*, 1994; Egloff *et al.*, 1995; Schubert *et al.*, 1995; Fauman *et al.*, 1996; Yang *et al.*, 1998), such a developmental signal transduction cascade may be involved in determining the color pattern.

Here we studied the effects of tungstate on the color-pattern determination in the Small Copper butterfly *Lycaena phlaeas daimio* (Lycaenidae, Lycaeninae). The induced modifications were examined in the light of spontaneous aberrant patterns as well as natural patterns of lycaenid butterflies. Our findings may contribute to elucidating an evolutionary mechanism for the color-pattern determination and speciation at least in Lycaenidae.

## Materials and methods

Adult individuals of the Small Copper butterfly *L. phlaeas daimio* (Lycaenidae, Lycaeninae) were caught in the Shonan-Hiratsuka campus of Kanagawa University, Japan. Eggs laid on hosting plants in a cage were then collected. Alternatively, live larvae or eggs were caught in the field. In both cases, larvae were fed on natural hosting plants at ambient temperature ( $25 \pm 5^\circ\text{C}$ ). Since it is very difficult to obtain any modified color-patterns without experimental manipulations, genetic differences among individuals including sex were ignored.

Based on the previous study (Otaki, 1998), we considered the sodium tungstate treatment to be functionally equivalent to but methodologically more efficient than the cold-shock treatment. Injections were made at a segmental boundary of pupae with 1.0 M sodium tungstate solution (0.2  $\mu\text{L}$ ) using a micropipette within 6 hours after pupation.

Induction rate (*IR*) for the treatment was calculated as follows:

$$IR(\%) = \frac{n_m}{N_e} \times 100$$

where  $N_e$  = the number of treated individuals with successful eclosion, and  $n_m$  = the number of modified individuals. The survival rate (*SR*) was calculated as follows:

$$SR(\%) = \frac{N_e}{N_t} \times 100$$

where  $N_t$  = the total number of treated individuals, and  $N_e$  = the number of treated individuals with successful eclosion.

Natural color-patterns of Japanese Lycaenid butterflies were compared to the modification patterns observed in *L. phlaeas daimio*, referring to Inoue *et al.* (1959) and other pictorial books of butterflies.

## Results and discussion

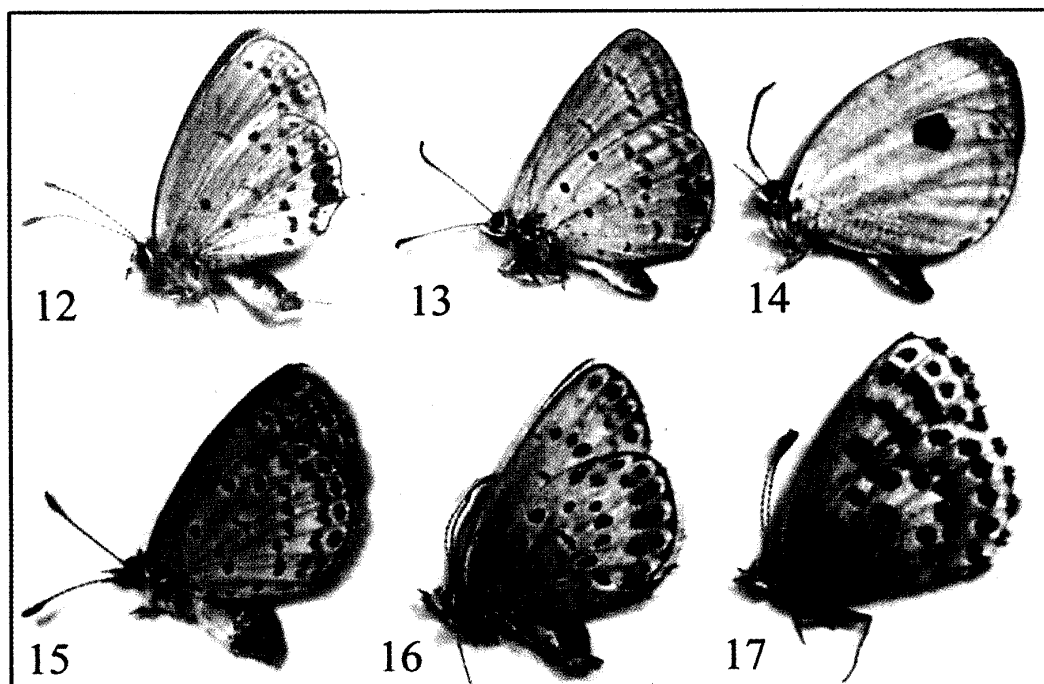
Among the treated individuals ( $n=29$ ), the survival rate was 72%, and its induction rate was 29%. This induction rate was much smaller than that of *V. cardui* (100%), although its survival rate was nearly comparable to that of *V. cardui* (ca 90%) (Otaki, 1998).

In the ventral forewings, many black spots enlarged in size and elongated along the wing cells in the affected individuals (Fig. 1). These spots are called “Media 1 (M1)” in the Schwanwitsch’s system (Schwanwitsch, 1949) or “distal band” of the central symmetry system in the Nijhout’s system (Nijhout, 1991). This is considered as the most diverse pattern element in Lycaenidae (Schwanwitsch, 1949; Nijhout, 1991). Similarly modified was “Externa 3 (E3)” (Schwanwitsch, 1949) or “element g” (Nijhout, 1991), which also shows high diversity in Lycaenidae (Schwanwitsch, 1949; Nijhout, 1991). These two elements often merged into the continuation in the tungstate-treated individuals.

In the ventral hindwings, three spots located close to the costal margin were most severely affected in response to the treatment. We also noted that there were modification variations not only among different spots but also among different individuals. This is not surprising, because similar response variations among individuals and pattern elements have frequently been observed in other species (Sakaguchi, 1981; Nijhout, 1984, 1985a; Otaki, 1998). In addition, the independence of each pattern elements has been known (Nijhout, 1985b; Nijhout & Wray, 1988; Paulsen & Nijhout, 1993).

It is worth while pointing out that in *V. cardui* the black spots diminished and in some cases completely disappeared (Otaki, 1998, 2003). This is in sharp contrast to *L. phlaeas daimio*, despite the fact that these two species have similar color patterns (*i.e.*, the black spots in the orange background).

Many aberrant butterflies caught in the field have been reported in Japanese lepidopterology journals, in which we found that some field-caught butterflies showed striking similarities to our experimentally modified butterflies (Figs 2–5). For example, an aberrant *L. phlaeas daimio* individual (Miyakuni, 1991) shows elongation of several spots only in the forewings (Fig. 3). Dorsal side of the wings are extensively melanized (Fig. 2), which was also seen in the tungstate-treated ones (not shown). Another two individuals of different species shown here (Fujiwara, 1992; Tsunemoto, 1988) have similar modification patterns (Figs 4–5). It is noteworthy that an aberrant *Tongeia fischeri* individual (Fig. 4) has severe modifications especially in the forewings. An aberrant *Pseudozizeeria maha argia* individual shows the same tendency in the forewings, and the black spots located at the costal margin are most severely affected in the hindwings (Fig. 5). These modification tendencies were all observed in the tungstate-treated individuals of *L. phlaeas daimio*, and thus they are likely to be seen widely at least in Lycaeninae and Polyommatainae, and possibly throughout Lycaenidae.



Figs 12–15. Comparison of natural color-patterns of various Lycaenid butterflies. 12. *Everes argiades hellotia*. 13. *Euchrysops cnejus*. 14. *Pithecops fulgens tsushimanus*. 15. *Pseudozizeeria maha okinawana*. 16. *Plebejus argus*. 17. *Scolitantides orion jezoensis*.

We reasoned that these modifications might have played an important role in speciation in Lycaenidae. An excellent example that supports this idea can be found in two *Maculinea* species, *M. teleius* and *M. arionides*. In comparing their color patterns, the “enlargement” or “elongation” of the black spots in the ventral forewings is notable in *M. arionides* but not in *M. teleius* (Figs 6–11). *M. arionides* is thus reminiscent of the *L. phlaeas daimio* individuals treated with tungstate. It is possible to speculate that since *M. arionides* lives in the higher altitudes in Japan (Fukuda *et al.*, 1984), the enlargement of the black spots in this species is somehow related to biological adaptation to lower temperature.

Natural forms of many other Lycaenid butterflies also show some tendencies observed in the treated *L. phlaeas daimio* (Figs 12–17). In *Euchrysops cnejus*, only a few black spots are especially dark in the ventral hindwings (Fig. 13), in contrast to *Everes argiades hellotia* (Fig. 12) and *Pseudozizeeria maha okinawana* (Fig. 15). These black spots correspond to those most severely affected after the tungstate treatment in *L. phlaeas daimio*. In *Pithecops fulgens tsushimanus*, only these spots exist in the ventral hindwings (Fig. 14). Comparison between *Plebejus argus* (Fig. 16) and *Scolitantides orion jezoensis* (Fig. 17) immediately reveals the difference of the spots in size throughout the ventral wings, as if *S. orion jezoensis* (Fig. 17) evolved after a genetic mutation of a gene being responsible for the color-pattern modifications induced by tungstate. These are just some of many examples that can be seen throughout Lycaenidae.

Accordingly, it is reasonable to speculate that there is a common mechanism for evolution of the color patterns and speciation in Lycaenidae. Since the color-patterns of Lycaenidae are considered to be similar to those of Nymphalidae (Schwanwitsch, 1949; Nijhout, 1991), the hypothetical mechanism for the color-pattern determination may be shared by these two families and possibly more widely in butterflies and moths. Although the polarity difference

in response to the tungstate treatment (*i.e.*, reduction versus enlargement of the black spots) are notable between *V. cardui* (Nymphalidae) and *L. phlaeas daimio* (Lycaenidae), this could simply result from a minor adjustment of the hypothetical developmental pathway for the color-pattern determination. The experimental results of the tungstate application to other families of butterflies will be reported elsewhere.

The formation of eyespots, which are local pattern elements, has been an intensive focus in evolutionary and developmental biology of butterflies (Nijhout, 1991; Brakefield *et al.*, 1996; Brunetti *et al.*, 2001; Beldade & Brakefield, 2002). On the other hand, a global or wing-wide regulatory mechanism for the color-pattern determination is almost entirely unknown at the molecular level. It is important to realize that phonocopies induced by experimental or spontaneous measures have their corresponding genetic mutations at least in the case of *Drosophila* and it has been speculated that just a few genes govern this type of modifications (Nijhout, 1984, 1985a; Otaki, 1998, 2003). This speculation encourages us to identify a target molecule for the putative “cold-shock hormone” and tungstate which might be responsible for the global coordination of the butterfly wing color-patterns. This line of research is also consistent with the heterochrony mechanism for the color-pattern determination (Takayama & Yoshida, 1997; Takayama *et al.*, 1997; Koch *et al.*, 2000a, 2000b).

### Acknowledgements

We are very grateful to Chouken Press, Ltd. (Osaka, Japan) for kindly giving us permission to reproduce several pictures of butterflies published in *Chouken Field*. For providing us beautiful pictures of *M. arionides*, we thank Mr Takuya Ito and Mr Ryo Takahashi in Hokkaido University. For permitting us to take pictures of natural specimens of *M. teleius* and *M. arionides*, we express our gratitude to Dr Masatoshi Takakuwa, Kanagawa Prefectural Museum of Natural History.

### References

- Barbera, A., Rodriguez-Gil, J. E. & J. J. Guinovart, 1994. Insulin-like actions of tungstate in diabetic rats. Normalization of hepatic glucose metabolism. *J. biol. Chem.* **269**: 20047–20053.
- Beldade, P. & P. M. Brakefield, 2002. The genetics and evo-devo of butterfly wing patterns. *Nat. Rev. Genet.* **3**: 442–452.
- Brakefield, P. M., 1987. Industrial melanism: do we have the answers? *Trends Ecol. Evol.* **2**: 117–122.
- Brakefield, P. M., Gates, J., Keys, D., Kesbeke, F., Wijngaarden, P. J., Monteiro, A., French, V. & S. B. Carroll, 1996. Development, plasticity and evolution of butterfly eyespot patterns. *Nature* **384**: 236–242.
- Brakefield, P. M. & T. G. Liebert, 2000. Evolutionary dynamics of declining melanism in the peppered moth in The Netherlands. *Proc.R. Soc. Lond. (B)* **267**: 1953–1957.
- Brunetti, C. R., Selegue, J. E., Monteiro, A., French, V., Brakefield, P. M. & S. B. Carroll, 2001. The generation and diversification of butterfly eyespot color patterns. *Curr. Biol.* **11**: 1578–1585.
- Egloff, M. P., Cohen, P. T., Reinemer, P. & D. Barford, 1995. Crystal structure of the catalytic subunit of human protein phosphatase 1 and its complex with tungstate. *J. mol. Biol.* **254**: 942–959.
- Fauman, E. B., Yuvaniyama, C., Schubert, H. L., Stuckey, J. A. & M. A. Saper, 1996. The X-ray crystal structures of *Yersinia* tyrosine phosphatase with bound tungstate and nitrate. Mechanistic implications. *J. biol. Chem.* **271**: 18780–18788.
- Fillat, C., Rodriguez-Gil, J. E. & J. J. Guinovart, 1992. Molybdate and tungstate act like vanadate on glucose metabolism in isolated hepatocytes. *Biochem. J.* **282**: 659–663.
- Fujiwara, T., 1992. Aberrant *Tongeia fischeri* caught in Tsushima. *Chouken Field* **7** (3): 31 (in Japanese).

- Fukuda, H., Hama, E., Kuzuya, T., Takahashi, A., Takahashi, M., Tanaka, B., Tanaka, H., Wakabayashi, M. & Y. Watanabe, 1984. *Maculinea teleius* and *Maculinea arionides*. *The Life Histories of Butterflies in Japan* 3: 262-269, pls 50-51. Hoikusha, Osaka. (In Japanese).
- Goto, Y., Kida, K., Ikeuchi, M., Kaino, Y. & H. Matsuda, 1992. Synergism in insulin-like effects of molybdate plus  $H_2O_2$  or tungstate plus  $H_2O_2$  on glucose transport by isolated rat adipocytes. *Biochem. Pharmacol.* **44**: 174-177.
- Grant, B., Owen, D. F. & C. A. Clarke, 1996. A parallel rise and fall of melanic peppered moths in America and Britain. *J. Heredity* **87**: 351-357.
- Haque, S. J., Flati, V., Deb, A. & B. R. Williams, 1995. Roles of protein-tyrosine phosphatases in Stat1 $\alpha$ -mediated cell signaling. *J. biol. Chem.* **270**: 25709-25714.
- Inoue, H., Okano, M., Shirozu, T., Sugi, S. & H. Yamamoto, 1959. *Iconographia Insectorum Japonicorum Colore naturali edita* 1. 284 pp., 184 pls. Hokuryukan, Tokyo. (In Japanese).
- Jiggins, C. D., Naisbit, R. E., Coe, R. L. & J. Mallet, 2001. Reproductive isolation caused by colour pattern mimicry. *Nature* **411**: 302-305.
- Kapan, D. D., 2001. Three-butterfly system provides a field test of mullerian mimicry. *Nature* **409**: 338-340.
- Koch, P. B., Behnecke, B. & R. H. French-Constant, 2000a. The molecular basis of melanism and mimicry in a swallowtail butterfly. *Curr. Biol.* **10**: 591-594.
- Koch, P. B., Lorenz, U., Brakefield, P. M. & R. H. French-Constant, 2000b. Butterfly wing pattern mutants: developmental heterochrony and co-ordinately regulated phenotypes. *Dev. Genes Evol.* **210**: 536-544.
- Li, J., Elberg, G., Gefel, D. & Y. Shechter, 1995. Permolybdate and pertungstate — potent stimulators of insulin effects in rat adipocytes: mechanism of action. *Biochemistry* **34**: 6218-6225.
- Matsumoto, J., 1994. Vanadate, molybdate and tungstate for orthomolecular medicine. *Med. Hypotheses* **43**: 177-182.
- Miyakuni, M., 1991. Melanic form of *Lycaena phlaeas* caught in the field. *Chouken Field* **6** (8): 35 (in Japanese).
- Nijhout, H. F., 1984. Colour pattern modification by coldshock in Lepidoptera. *J. Embryol. exp. Morphol.* **81**: 287-305.
- , 1985a. Cautery-induced colour patterns in *Precis coenia* (Lepidoptera: Nymphalidae). *J. Embryol. exp. Morphol.* **86**: 191-203.
- , 1985b. Independent development of homologous pattern elements in the wing patterns of butterflies. *Dev. Biol.* **108**: 146-151.
- , 1991. *The Development and Evolution of Butterfly Wing Patterns*. 297 pp., 8 pls. Smithsonian Institution Press, Washington.
- Nijhout, H. F. & G. A. Wray, 1988. Homologies in the color patterns of the genus *Heliconius* (Lepidoptera, Nymphalidae). *Biol. J. Lin. Soc.* **33**: 345-365.
- Otaki, J. M., 1998. Color-pattern modifications of butterfly wings induced by transfusion and oxyanions. *J. Insect Physiol.* **44**: 1181-1190.
- , 2003. Asymmetrical color pattern of *Vanessa cardui*: a case report of a field-caught individual and experimental pattern modifications. *Butterflies* (35): 50-56.
- Paulsen, S. M. & H. F. Nijhout, 1993. Phenotypic correlation structure among elements of the color pattern in *Precis coenia* (Lepidoptera: Nymphalidae). *Evolution* **47**: 593-618.
- Sakaguchi, K., 1981. *Insects of the World* **5**: 194-197, pl. 49. Hoikusha, Osaka. (In Japanese).
- Schubert, H. L., Fauman, E. B., Stuckey, J. A., Dixon, J. E. & M. A. Saper, 1995. A ligand-induced conformational change in the *Yersinia* protein tyrosine phosphatase. *Protein Sci.* **4**: 1904-1913.
- Schwanwitsch, B. N., 1949. Evolution of the wing-pattern in the Lycaenid Lepidoptera. *Proc. zool. Soc.* **119**: 189-263.
- Sibatani, A., 1980. Wing homoeosis in Lepidoptera: A survey. *Dev. Biol.* **79**: 1-18.
- , 1983a. A compilation of data on wing homoeosis in Lepidoptera. *J. Res. Lepid.* **22**: 1-46.
- , 1983b. A compilation of data on wing homoeosis in Lepidoptera: supplement I. *J. Res. Lepid.* **22**: 118-125.
- Stuckey, J. A., Schubert, H. L., Fauman, E. B., Zhang, Z. Y., Dixon, J. E. & M. A. Saper, 1994. Crystal



- structure of *Yersinia* protein tyrosine phosphatase at 2.5 Å and the complex with tungstate. *Nature* **370**: 571–575.
- Sweeney, A., Jiggins, C. & S. Johnsen, 2003. Polarized light as a butterfly mating signal. *Nature* **423**: 31–32.
- Takayama, E. & A. Yoshida, 1997. Color pattern formation on the wing of a butterfly *Pieris papae*. 1. Cautery induced alteration of scale color and delay of arrangement formation. *Dev. Growth Diff.* **39**: 23–31.
- Takayama, E., Motoyama, M. & A. Yoshida, 1997. Color pattern formation on the wing of a butterfly *Pieris papae*. 2. Color determination and scale development. *Dev. Growth Diff.* **39**: 485–491.
- Tsunemoto, A., 1988. Aberrant *Pseudozizeeria maha*. *Chouken Field* **3** (9): 9, pl. 5 (in Japanese).
- Uesugi, K., 1991. Temporal change in records of the mimetic butterfly *Papilio polytes* with establishment of its model *Pachliopta aristolochiae*. *Jap. J. Ent.* **59**: 183–198.
- Warren, R. W., Nagy, L., Selegue, J., Gates, J. & S. Carroll, 1994. Evolution of homeotic gene regulation and function in flies and butterflies. *Nature* **372**: 458–461.
- Yang, J., Liang, X., Niu, T., Meng, W., Zhao, Z. & G. W. Zhou, 1998. Crystal structure of the catalytic domain of protein-tyrosine phosphatase SHP-1. *J. biol. Chem.* **273**: 28199–28207.

## 摘 要

シジミチョウ科蝶類における色彩パターン修飾と種分化 (大瀧丈二・山本晴彦)

蛋白質チロシン・ホスファターゼ阻害剤であるタングステン酸ナトリウムを蛹化直後の蛹に注射すると、翅の色彩パターン修飾が見られることがヒメアカタテハにおいて示されている。これは、鱗粉細胞において、受容体型蛋白質チロシン・キナーゼによって開始される細胞内情報伝達経路が長時間活性化されることに起因すると考えられる。この論文では、ベニシジミを用いて同様の実験を行った。黒斑が縮小・消滅するヒメアカタテハとは対照的に、ベニシジミでは特に前翅裏面の黒斑列が拡大した。このような修飾は、ベニシジミばかりでなく、クロツバメシジミやヤマトシジミなど多くのシジミチョウ科蝶類においても野外で頻繁に観察されている。正常な日本の蝶の色彩パターンにおいても同様の傾向が観察された。小さな黒斑を持つゴマシジミと細長く大きな黒斑を持つオオゴマシジミとの相違はその最も顕著な例である。このような知見は、少なくともシジミチョウ科蝶類では、鱗粉細胞の分化に関わる仮説的な細胞内情報伝達経路の修飾が種分化に深く関連していることを示している。

(Accepted May 3, 2003)